World Journal of Microbiology and Biotechnology (2025) 41:141
https://doi.org/10.1007/s11274-025-04356-0

RESEARCH

# Co-selection of antibiotic resistance and heavy metal tolerance in bacterial species isolated from automobile scrapyard soils

**F. A. Jimoh[1] · A. T. Ajao[1] · W. T. Aborisade[1] · Z. B. Abdulsalam[1]**

Received: 4 December 2024 / Accepted: 6 April 2025
© The Author(s), under exclusive licence to Springer Nature B.V. 2025

## Abstract

This study investigates the physicochemical properties, heavy metal concentrations, and bacterial profiles of contaminated scrapyard soils compared to fertile agricultural soil. The results indicate that scrapyard soils exhibit higher bulk density (1.10 1.30 g/cm³), lower organic carbon (0.80 1.10%), and reduced organic matter (1.37 1.90%) levels, suggesting deterioration in soil fertility. Electrical conductivity is significantly elevated (0.70 0.80 dS/m), indicating increased ionic strength. Heavy metal analysis reveals significantly higher concentrations of Pb (14 18 mg/kg), Cd (3 7 mg/kg), Zn (20 40 mg/kg), Cu (23 35 mg/kg), Ni (23 30 mg/kg), and As (3.85 5.5 mg/kg). Bacterial loads in scrapyard soils (3.08 3.76 Log CFU/g) are considerably lower than in control soil (7.88 Log CFU/g), alongside decreased soil enzyme activities, including dehydrogenase (130 170 µg TPF/g/h), urease (110 130 µg $NH_4^+$/g/h), phosphatase (70–90 µg pNP/g/h), and catalase (85–95 µg $O_2$/g/h). The study isolates *Sphingomonas paucimobilis*, *Klebsiella aerogenes*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas reactans*, and *Bacillus subtilis*, which exhibit varying resistance to antibiotics and heavy metals. A strong correlation is observed between heavy metal tolerance and antibiotic resistance ($r = 0.78$ 0.88, $P < 0.05$), suggesting a synergistic effect where metal exposure enhances bacterial resilience to antibiotics. Notably, the MICs of tetracycline and ampicillin increase substantially in Cd-exposed isolates (up to 35 µg/mL and 25 µg/mL, respectively), while Ni exposure in *Pseudomonas reactans* results in a two-fold increase in the MIC of chloramphenicol (from 10.5 µg/mL to 20 µg/mL). The correlation between soil enzyme activity and dominant bacterial species further highlights the influence of heavy metal contamination on microbial function. These findings underscore the environmental risks posed by scrapyard contamination and the adaptive mechanisms of multi-resistant bacteria in response to heavy metal stress.

**Keywords** Heavy metal tolerance · Antibiotic resistance · Scrapyard soil · Multi-resistant bacteria · Physicochemical properties

## Introduction

The rapid industrialization and urbanization of recent decades have led to the proliferation of automotive scrapyards (Guilin 2015; Ibe et al. 2020), which are essential for recycling and waste management. However, these scrapyards often pose significant environmental risks due to the accumulation of heavy metals and other pollutants. Heavy metals such as cadmium, nickel, lead, and chromium are known to persist in the environment and can adversely affect soil health, microbial communities, and potentially human health through the food chain (Mlinarics et al. 2009). In these contaminated environments, bacteria can evolve traits that confer tolerance to heavy metals and resistance to antibiotics, leading to complex ecological and public health challenges (Chen et al. 2019; Zhou et al. 2022; Gillieatt and Coleman 2024). Automotive scrapyards are recognized as hotspots for heavy metal contamination, which can create selective pressures on bacterial populations, leading to the development of metal-tolerant and antibiotic-resistant strains (Barbosa et al., 2022; Liu et al. 2024). Despite the known environmental impacts, there is a lack of comprehensive studies investigating the simultaneous expression of heavy metal tolerance and antibiotic resistance traits in bacteria isolated from these environments (Najar et al. 2022). This knowledge gap limits our understanding of how

✉ F. A. Jimoh
fausat.jimoh@kwasu.edu.ng

[1] Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria

Published online: 28 April 2025

such co-expressions might contribute to microbial adaptation and potentially exacerbate public health risks. The aim of this study is to assess the co-expression of heavy metal tolerance and antibiotic resistance traits in bacteria isolated from automotive scrapyards. This involves evaluating how bacterial isolates from these environments tolerate heavy metals and resists antibiotics, and determining the relationship between these two traits. Extensive research has been conducted on the co-occurrence of heavy metal contamination and antibiotic resistance in various environments, including soil (Wang et al. 2021; Edet et al. 2023; Amarasekara et al. 2023). The mechanisms of co-selection, horizontal gene transfer, and environmental persistence of multi-resistant bacteria in metal-contaminated sites has been well documented (Vats et al. 2022; Engin et al. 2023). While studies have investigated either metal tolerance or antibiotic resistance, limited research has examined the co-expression of these traits within the same bacterial isolates from automotive scrapyards (Pal et al. 2017; Maurya et al. 2020). These environments are characterized by high levels of mixed metal pollutants and oil-derived contaminants, yet their influence on microbial dynamics remains underexplored. Therefore, this study is intended to contribute to existing knowledge by examining bacterial adaptation to heavy metal stress in scrapyard soils and its implications for antibiotic resistance development, providing insights into their environmental and public health implications.

This research gap limits a comprehensive understanding of the ecological and health implications of heavy metal tolerance and antibiotic resistance in bacteria from automotive scrapyards. Assessing their environmental impact and potential risks is crucial, as bacterial adaptation in contaminated environments can influence remediation efforts and environmental management. This study provides valuable insights into these adaptation mechanisms, aiding in the development of effective strategies for monitoring and controlling bacterial resistance in industrial waste-impacted environments. By identifying bacteria that exhibit both traits, the findings help predict potential risks associated with antibiotic-resistant pathogens. Addressing this research gap is essential for safeguarding public health and enhancing environmental protection. This study aims to: (1) Investigate the co-selection of heavy metal tolerance and antibiotic resistance in bacteria isolated from automotive scrapyards. (2) Assess the correlation between metal contamination levels and bacterial resistance profiles. (3) Evaluate the potential environmental and public health risks associated with metal-resistant and antibiotic-resistant bacterial populations. Addressing this gap is essential for safeguarding public health and enhancing environmental protection.

## Materials and methods

### The sampling sites

The study was conducted at an automotive scrapyard in Oloje, Ilorin, Kwara State, which specializes in the collection, storage, and sale of used car parts and scrap metal. The soil samples were randomly collected at a depth of 0–25 cm from several locations within the scrapyard using the clean soil auger: near the entrance, areas with a high density of car parts, and the site perimeter. Additional samples were taken from areas where car fluids, such as oil and coolant, are commonly spilled. The dry soil samples collected were entirely placed in clean, labelled polyethylene bags and then transported to the laboratory for further analysis. The soil samples were grinded using mortar and pestle to reduce the particle size and then sieved through a 2 mm mesh to obtain acceptable and homogeneous samples. Each sample was divided into two, and the first part stored at room temperature for physicochemical and enzyme analyses as described by Li et al. (2015).

### Physicochemical analysis

In this study, various analytical methods were employed to assess soil physical, chemical, and biological properties, as well as heavy metal concentrations. Physicochemical and Heavy Metal Analysis of Soil The pH of the soil samples was determined using a digital pH meter in 1.0:2.5 soil-water suspension, particle size distribution was determined using the hydrometer method (Amos et al. 2023), Cation Exchange Capacity (CEC) was determined by saturating the samples with sodium acetate(Aprile and Lorandi 2012), Electrical conductivity (EC) was determined in 1.0:2.5 soil-water saturation and organic matter using WalkleyBlack method as described by Bilgili et al. (2010) while the sulphate and phosphate concentrations of the soils were determined according to the method by Amos et al. (2023). Heavy metal analysis of the samples was carried out using Inductively Coupled Plasma Atomic Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES Thermo Fisher ICAP 6300). Microbial population was estimated using the serial dilution plate count method (Fawole and Oso 2001).

### Isolation of heavy metal-tolerant bacteria from scrapyard soil

#### Chemicals and media

Stock solutions of heavy metals (1,000 mg/L) were prepared using cadmium chloride ($CdCl_2$), lead nitrate ($Pb(NO_3)_2$), potassium dichromate ($K_2Cr_2O_7$), and nickel sulfate ($NiSO_4$)

(Merck, Germany). These stock solutions were sterilized by filtration (0.22 µm) and stored at 4 °C until use. Nutrient agar and Luria-Bertani (LB) medium were used for the growth and isolation of microorganisms. The culture media were supplemented with 50 mg/L of the respective heavy metals after autoclaving and allowed to cool to 45–50 °C before being poured into sterile Petri dishes. Scrapyard soil samples were serially diluted in sterile saline water, with 9 mL of saline in each of six test tubes. One milliliter of soil sample was added to the first test tube to achieve a $10^{-1}$ dilution, with serial dilutions performed up to $10^{-6}$. Then, 0.1 mL of each dilution was spread onto the metal-supplemented agar plates and incubated at 36–37 °C for 2 days. Colonies with differing morphological appearances were selected for further studies and subcultured on the same media ((Mustapha and Halimoon 2015).

## Enzymatic activities

### Urease activity

A soil sample of about 5 g had mixed with 5 mL of toluene, 20 mL of distilled water, and 10 mL of 10% urea solution. The mixture was incubated at 37 ◦C for 24 h. After incubation, the solution was centrifuged for 5 min at 4000 rpm, 1 mL aliquot was mixed with 4 mL of sodium phenol solution (containing 100 mL of 6.6 M phenol solution and 100 mL of 6.8 M NaOH) and 3 mL of 0.9% sodium hypochlorite solution. The released ammonium in the solution was quantified colorimetrically at 578 nm on a spectrophotometer, and the urease activity was expressed as µgNH4N g − 1 soil $h^{-1}$ (Liu and Tan 2014).

### Phosphatase activity

The reaction mixture of 5 g soil, five drops of toluene, 10 mL of distilled water, and 10 mL of disodium phenyl phosphate solution had used to analyse phosphatase activity. The suspension was incubated at 37 ◦C for 24 h and centrifuged for 5 min at 4000 rpm. To add colour to the supernatant, a 0.25 mL of ammonia-ammonium chloride buffer, at pH 9.6, 0.5 mL of 2% 4-amino antipyrine, and 0.5 mL of 8% potassium ferrocyanide was added to the solution. The phenol content was determined colorimetrically at 510 nm on a spectrophotometer. Phosphatase activity was expressed as µgphenol g − 1 soil $h^{-1}$ (Tan et al. 2014).

### Catalase activity

A 5 g of soil sample had mixed with 0.5 mL of toluene into a conical flask. The solution was stored at 4 ◦C for 30 min, after which a 5 mL of 3% $H_2O_2$ solution was added and stored again at 4 ◦C for 1 h. Afterward, 2 M of $H_2SO_4$ was added to the solution, and then the suspension was titrated with 0.01 M of $KMnO_4$ until a faint pink colour appears in the solution. Catalase enzyme activity was expressed as mL of $KMnO_4$ g − 1 soil $h^{-1}$ (Tan et al. 2014).

Dehydrogenase activity is determined by reducing 2,3,5-triphenyl tetrazolium chloride (TTC), extracting the triphenyl formazan (TPF), and measuring as 485 nm (Yeboah et al. 2021).

## Antibiotic susceptibility testing

The susceptibility of bacterial isolates to five antibiotics Cefotaxime (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), and Colistin (10 µg) was tested using the Kirby-Bauer disc diffusion method. Following CLSI guidelines (2018), bacterial suspensions were adjusted to a 0.5 McFarland standard ensuring a uniform concentration of approximately $1 \times 10^8$ CFU/mL and inoculated onto Mueller-Hinton agar plates. After placing antibiotic discs on the agar surface, plates were incubated at 25 ± 2 °C for 24 h. The zones of inhibition were measured, and isolates were classified as resistant, intermediate, or sensitive based on established threshold values (Yao et al. 2021).

## Heavy metal susceptibility testing

Bacterial isolates, adjusted to a 0.5 McFarland standard, were spread onto Mueller-Hinton agar plates after incubation in LB broth with sub-lethal concentrations of lead (5 mg/L), cadmium (1 mg/L), and nickel (2 mg/L). Using a 6 mm cork borer, wells were created in the agar, and varying concentrations of heavy metals were added. Plates were incubated at 37 °C for 24 h. Zones of inhibition were measured to determine susceptibility, following established protocols (AL-Sa'ady et al. 2014).

## Determination of minimum inhibitory concentration of heavy metals

The MIC of heavy metals Cadmium (Cd), Lead (Pb), and Nickel (Ni) against bacterial isolates was determined by preparing stock solutions ranging from 50 to 200 mg/mL. These were diluted in Luria-Bertani (LB) broth to achieve sub-lethal concentrations (e.g., Cadmium at 5 mg/mL, Lead at 50 mg/mL, and Nickel at 2 mg/mL). Bacterial isolates were inoculated into sterile LB broth containing these diluted metal solutions and incubated at 37 °C with shaking for 16–24 h. Control tubes containing LB broth without metal were also prepared. MIC was identified as the lowest concentration that inhibited visible bacterial growth. This

method follows standard protocols as outlined in previous studies (Ntabugi et al. 2021).

### Determining minimum inhibitory concentration (MIC) of antibiotics

Stock solutions of Tetracycline, Ampicillin, and Chloramphenicol were prepared at concentrations ranging from 0.5 µg/mL to 50 µg/mL. Bacterial isolates, suspended in normal saline and adjusted to a 0.5 McFarland standard, were inoculated onto Mueller-Hinton agar plates. Antibiotic discs were placed on the agar, and the plates were incubated at 37 °C for 16–18 h. The MIC was identified as the lowest concentration that inhibited visible bacterial growth, following standard protocols (Ayalew 2021).

### Combined assay for heavy metal exposure and antibiotic resistance

To assess the impact of sub-lethal heavy metal exposure on the antibiotic resistance of bacterial isolates, stock solutions of lead (Pb) at 50 mg/L, cadmium (Cd) at 10 mg/L, and nickel (Ni) at 20 mg/L were prepared. These were diluted in Luria-Bertani (LB) broth to achieve sub-lethal concentrations (Pb at 5 mg/L, Cd at 1 mg/L, Ni at 2 mg/L). Bacterial isolates were inoculated into LB broth containing these metals, with a control set grown in LB broth without heavy metals. After 16–24 h of incubation at 37 °C, cultures were spread on Mueller-Hinton agar plates, followed by the application of antibiotic discs (ampicillin at 10 µg, tetracycline at 30 µg, chloramphenicol at 30 µg). Plates were incubated at 37 °C for 16–18 h. Zones of inhibition were measured to assess changes in antibiotic susceptibility due to heavy metal exposure, following the protocol described by Lin et al. (2019)

### Statistical analysis

The data shown in the Tables were means (X) and standard errors (S.E). The results were subjected to Analysis of variance (ANOVA) using SPSS version 20.0. Duncan multiple range test was used to separate their means. Data analysis involved calculating the correlation between MIC values for heavy metals and resistance levels to antibiotics. Statistical analysis, such as the Pearson correlation coefficient, was performed to assess the strength and significance of the correlation, and to analyze if pre-exposure to heavy metals increased antibiotic resistance in the isolates.

## Results

Table 1 reveals that contaminated soil from the scrapyard has higher bulk density (1.10–1.30 g/cm³) and lower organic carbon (0.80–1.10%) and organic matter (1.37–1.90%) compared to fertile agricultural soil. The pH of the contaminated soil is slightly acidic (5.6-6.0), while its electrical conductivity is significantly higher (0.70–0.80 dS/m). Contaminated soil also shows elevated levels of sulphate (24–27 mg/kg) and phosphate (8–11 mg/kg) but lower Kjeldahl nitrogen (0.27–0.34 g/kg). Additionally, it has a higher sand content and lower clay content compared to the fertile soil.

Table 2 shows that heavy metal concentrations in scrapyard soils are significantly higher than in agricultural soil controls. Lead (Pb) ranges from 14 to 18 mg/kg in the scrapyard soils, compared to 4.7 mg/kg in the control. Cadmium (Cd) levels are 3 to 7 mg/kg, much higher than 0.5 mg/kg in the control. Zinc (Zn) and Copper (Cu) concentrations are also elevated, with Zn ranging from 20 to 40 mg/kg and Cu from 23 to 35 mg/kg, compared to 7.5 mg/kg and 5.35 mg/kg in the control, respectively. Nickel (Ni) and Arsenic (As) levels are higher in scrapyard soils, with Ni ranging from 23 to 30 mg/kg and As from 3.85 to 5.5 mg/kg, compared to 6.21 mg/kg and absent in the control. Chromium (Cr) is

**Table 1** Physicochemical parameters of contaminated soil in scrapyard

| Parameter | Sampling Sites | | | | | Fertile Agricultural Soil |
|---|---|---|---|---|---|---|
| | OJ-1 | OJ-2 | OJ-3 | OJ-4 | OJ-5 | |
| Soil Bulk Density (g/cm³) | 1.25±0.2 | 1.20±0.3 | 1.30±0.2 | 1.18±0.4 | 1.10±0.2 | 0.85±0.00 |
| Organic Carbon (%) | 0.95±0.1 | 1.00±0.2 | 1.10±0.3 | 0.85±0.2 | 0.80±0.1 | 1.50±0.1 |
| Organic Matter (%) | 1.65±0.1 | 1.72±0.3 | 1.90±0.4 | 1.47±0.3 | 1.37±0.2 | 2.59±0.2 |
| pH | 5.8±0.2 | 6.0±0.3 | 5.9±0.2 | 5.7±0.4 | 5.6±0.2 | 6.50±0.2 |
| Electrical Conductivity (dS/m) | 0.75±0.1 | 0.78±0.2 | 0.80±0.1 | 0.70±0.2 | 0.72±0.1 | 50.00±4.0 |
| Sulphate (mg/kg) | 25±2.55 | 27±3.41 | 26±2 | 24±1 | 25±3 | 1.50±0.1 |
| Phosphate (mg/kg) | 9±1 | 11±2 | 10±1 | 8±1 | 9±1 | 3.00±0.2 |
| Kjeldahl Nitrogen (K) (g/kg) | 0.30±0.02 | 0.27±0.01 | 0.34±0.03 | 0.28±0.02 | 0.32±0.02 | 0.50±0.03 |
| Sand (%) | 55±3 | 54±2 | 56±3 | 52±2 | 53±3 | 65.00±5.0 |
| Silt (%) | 22±2 | 20±1 | 21±2 | 23±1 | 22±2 | 20.00±3.0 |
| Clay (%) | 11.40±4.3 | 8.91±2.0 | 11.95±6.0 | 11.83±4.5 | 12.74±2.3 | 15.00±2.0 |

**Table 2** Heavy metal concentrations in scrap yard soils

| Parameter (mg/kg) | Sampling Sites | | | | | Control (Agricultural Soil) |
|---|---|---|---|---|---|---|
| | OJ-1 | OJ-2 | OJ-3 | OJ-4 | OJ-5 | |
| Lead (Pb) | 22±10 | 16±15 | 18±20 | 14±10 | 15±1.52 | 4.7±0.21 |
| Cadmium (Cd) | 5±0.45 | 6±0.55 | 7±1.38 | 4±0.53 | 3±0.31 | 0.5±0.18 |
| Zinc (Zn) | 30±2.20 | 35±1.25 | 40±2.30 | 25±1.59 | 20±2.24 | 7.5±5.11 |
| Copper (Cu) | 20±2.55 | 35±3.72 | 27±2.10 | 23±2.50 | 33±3.99 | 5.35±1.75 |
| Nickel (Ni) | 20±3.90 | 25±2.20 | 30±3.50 | 27±3.11 | 23±1.78 | 6.21±1.90 |
| Chromium (Cr) | 5±0.37 | 3.0±0.00 | 3.5±0.66 | 3.0±0.22 | 2.5±0.00 | - |
| Arsenic (As) | 7±1.85 | 5.11±1.27 | 4.35±0.42 | 3.85±0.45 | 5.5±0.25 | - |
| Iron (Fe) | 75±3.15 | 65±6.52 | 58±5.81 | 72±7.35 | 81±8.52 | 8.1±0.01 |

**Table 3** Bacterial loads and soil enzymes diversity in scrapyard soils (Mean±Standard Error)

Superscript letters (a, b, c, d) indicate significant differences between means in the same column ($P<0.05$).Duncans Multiple Range Test (DMRT) was used to compare mean values

| Sampling Site | Metal-Tolerant Bacterial Count ($Log_{10}$CFU/g) | Dehydrogenase (µg TPF/g/h) | Urease (µg $NH_4^+$/g/h) | Phosphatase (µg pNP/g/h) | Catalase (µg $O_2$/g/h) |
|---|---|---|---|---|---|
| **OJ-1** | $3.08\pm0.21^{c}$ | $150\pm5.47^{c}$ | $120\pm2.16^{bc}$ | $80\pm1.92^{bc}$ | $90\pm2.27^{b}$ |
| **OJ-2** | $2.93\pm0.00^{c}$ | $130\pm2.85^{d}$ | $110\pm3.30^{c}$ | $75\pm2.52^{c}$ | $85\pm4.71^{b}$ |
| **OJ-3** | $3.76\pm0.11^{b}$ | $140\pm3.11^{c}$ | $115\pm1.55^{bc}$ | $70\pm4.49^{c}$ | $88\pm3.50^{b}$ |
| **OJ-4** | $3.26\pm0.15^{b}$ | $160\pm6.45^{b}$ | $125\pm3.95^{b}$ | $85\pm4.00^{b}$ | $92\pm3.87^{b}$ |
| **OJ-5** | $2.30\pm0.00^{d}$ | $170\pm4.40^{b}$ | $130\pm2.75^{b}$ | $90\pm3.22^{b}$ | $95\pm2.36^{b}$ |
| **Control** | $7.88\pm0.30^{a}$ | $345\pm5.44^{a}$ | $212\pm2.79^{a}$ | $130\pm5.33^{a}$ | $115\pm2.71^{a}$ |

**Table 4** Resistance and sensitivity profiles of bacterial isolates to antibiotics and heavy metals

| Substance | Threshold Values (mm) | | Isolates | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Zone of Inhibitions | | | | | |
| Antibiotic | Resistant (*R*) | Sensitive (S) | OM-1 | OM-2 | OM-3 | OM-4 | OM-5 | OM-6 |
| **Ampicillin** | ≥32 | ≤8 | R | S | R | S | R | S |
| **Tetracycline** | ≥16 | ≤4 | R | R | S | R | R | S |
| **Chloramphenicol** | ≥32 | ≤8 | R | S | R | S | R | S |
| **Heavy Metals** | | | | | | | | |
| **Cadmium** (Cd) | ≥20 | ≤5 | R | S | R | S | R | S |
| **Lead** (Pb) | ≥15 | ≤3 | S | R | R | S | R | S |
| **Nickel** (Ni) | ≥10 | ≤2 | R | S | R | R | S | R |

R = Resistant; S = Sensitive. Threshold values are expressed in millimeters (mm) for the zone of inhibition. Values indicate the minimum diameter for categorizing resistance (R) and sensitivity (S) based on established guidelines

lower in scrapyard soils, ranging from 2.5 to 3.5 mg/kg, compared to 5 mg/kg in the control. Iron (Fe) concentrations are variable but generally lower in scrapyard soils compared to the control.

Table 3 presents data on bacterial loads and soil enzyme activities across different scrapyard soil sites compared to a control. Metal-tolerant bacterial counts range from 3.08 Log CFU/g to 3.76 Log CFU/g in scrapyard soils, significantly lower than 7.88 Log CFU/g in the control. Dehydrogenase activity varies from 130 to 170 µg TPF/g/h in scrapyard soils, compared to 345 µg TPF/g/h in the control. Urease activity is lower in scrapyard soils, ranging from 110 to 130 µg $NH_4^+$/g/h, compared to 212 µg $NH_4^+$/g/h in the control. Phosphatase activity ranges from 70 to 90 µg pNP/g/h in scrapyard soils, while the control shows 130 µg pNP/g/h. Catalase activity ranges from 85 to 95 µg $O_2$/g/h in scrapyard soils, lower than 115 µg $O_2$/g/h in the control.

Table 4 shows the resistance and sensitivity profiles of bacterial isolates to various antibiotics and heavy metals. For antibiotics, OM-1 and OM-3 are resistant to Ampicillin, while OM-2, OM-4, and OM-6 are sensitive. OM-1, OM-2, OM-4, and OM-5 show resistance to Tetracycline, with OM-3 and OM-6 being sensitive. Chloramphenicol resistance is observed in OM-1 and OM-3, while OM-2, OM-4, and OM-6 are sensitive. Regarding heavy metals, OM-1 and OM-3 are resistant to Cadmium (Cd), while OM-2, OM-4, and OM-6 are sensitive, while, Lead (Pb), OM-1 and OM-4 are sensitive, whereas OM-2, OM-3, and OM-6 are resistant. Nickel (Ni) resistance is found in OM-1, OM-3, and OM-5, with OM-2, OM-4, and OM-6 being sensitive. The screening of bacterial isolates for metal tolerance showed varied growth responses across heavy metal concentrations. Some isolates grew well at high levels of Cadmium (Cd), Lead (Pb), and Nickel (Ni), while others were inhibited at lower levels. Tolerance was observed up to 100 mg/L

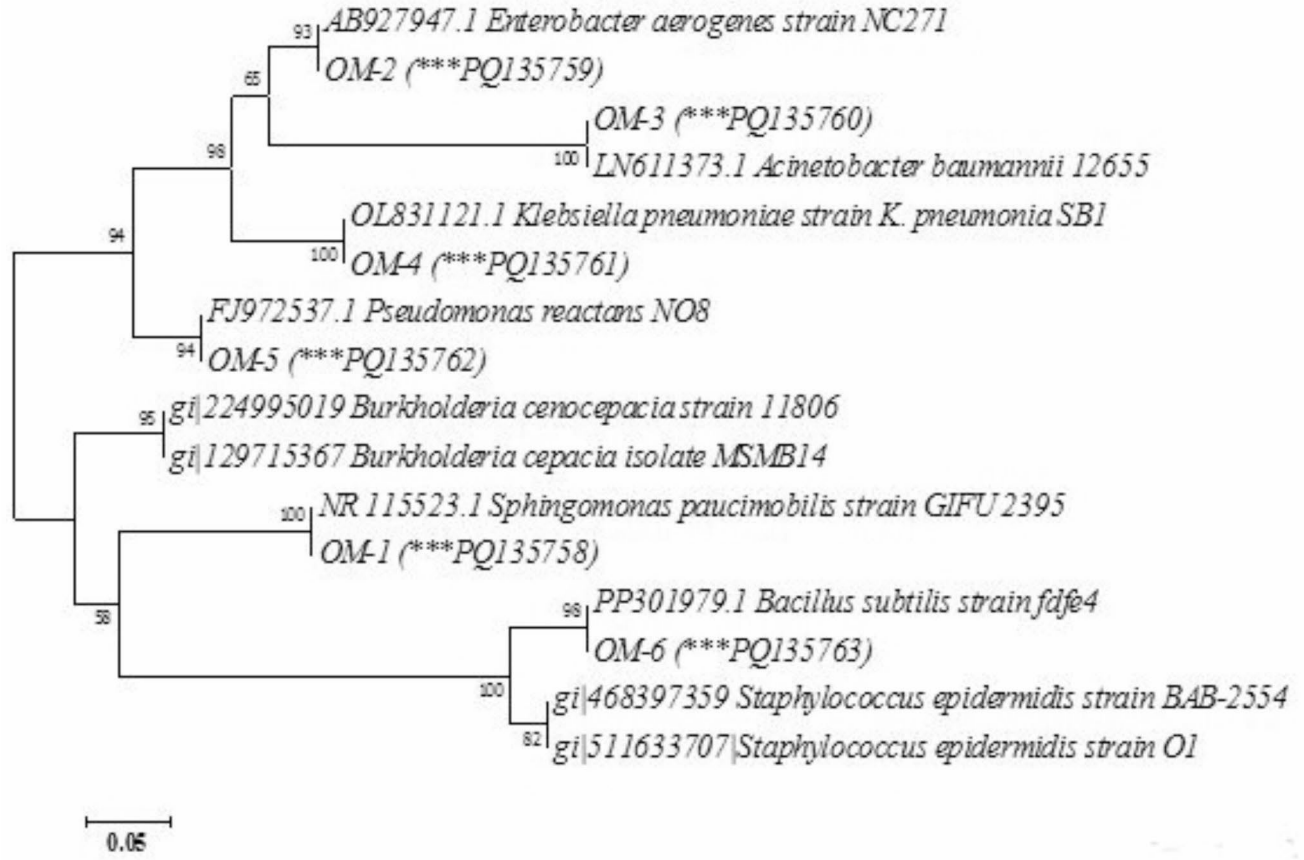



**Fig. 1** Phylogenetic tree of Metal Tolerant Bacteria isolated from the scrapyard constructed by Neighbor Joining Method. Number in the parenthesis represent GenBank Accession Numbers

**Table 5** MIC values for heavy metals and antibiotics resistance

| Isolate | Cadmium | Lead | Nickel | Tetracycline | Ampicillin | Chloramphenicol |
|---|---|---|---|---|---|---|
| | (MIC, µg/mL) | | | (MIC, µg/mL) | | |
| OM-1 | $150 \pm 10.11^{a}$ | $100 \pm 8.21^{b}$ | $75 \pm 5.10^{c}$ | $35 \pm 1.57^{a}$ | $25 \pm 1.31^{b}$ | $30 \pm 2.62^{a}$ |
| OM-2 | $90 \pm 7.43^{a}$ | $50 \pm 5.30^{b}$ | $50 \pm 4.33^{b}$ | $7.5 \pm 0.90^{a}$ | $4.5 \pm 0.75^{b}$ | $7.5 \pm 0.86^{a}$ |
| OM-3 | $130 \pm 9.22^{a}$ | $90 \pm 6.18^{b}$ | $70 \pm 5.82^{c}$ | $25 \pm 2.28^{a}$ | $20 \pm 1.28^{a}$ | $15 \pm 1.75^{a}$ |
| OM-4 | $90 \pm 10.88^{a}$ | $50 \pm 7.26^{b}$ | $65 \pm 5.37^{b}$ | $5 \pm 1.66^{a}$ | $7.5 \pm 1.75^{a}$ | $10.5 \pm 1.16^{a}$ |
| OM-5 | $140 \pm 11.45^{a}$ | $90 \pm 8.07^{b}$ | $70 \pm 6.11^{c}$ | $20 \pm 0.85^{a}$ | $12.5 \pm 1.34^{b}$ | $10.5 \pm 0.25^{b}$ |
| OM-6 | $80 \pm 8.45^{a}$ | $40 \pm 5.23^{c}$ | $55 \pm 5.91^{b}$ | $5.25 \pm 0.30^{a}$ | $7.5 \pm 0.45^{a}$ | $5.50 \pm 0.31^{a}$ |

Superscript letters (a, b, c, d) indicate significant differences between means in the same column ($P < 0.05$).Duncans Multiple Range Test (DMRT) was used to compare mean values

for Cadmium, 50 mg/L for Lead, and up to 150 mg/L for Nickel, with some isolates thriving at high Nickel concentrations. These findings suggest that isolates demonstrating high tolerance, especially to Nickel, may be suitable for bioremediation applications.

## Identification of isolates

The isolates were identified using 16 S rRNA gene sequencing. The results revealed that the majority of the isolates belonged to the genera *Pseudomonas* (50%), *Bacillus* (30%), and *Acinetobacter* (20%) (Fig. 1).

Table 5 reveals the MIC values for heavy metals and antibiotics among bacterial isolates. OM-1 and OM-5 have the highest tolerance to Cadmium, while OM-6 shows the lowest. OM-2 demonstrates the greatest tolerance to Lead, whereas OM-6 has the lowest. Nickel tolerance is highest in OM-1 and OM-5. For antibiotics, OM-2 exhibits the highest resistance to Tetracycline and Ampicillin, while OM-1 shows the lowest resistance. OM-6 has the highest

**Table 6** Correlation and statistical analysis

| Parameter Combination | Correlation Coefficient ($r$) | $p$-value |
|---|---|---|
| Cadmium vs. Tetracycline Resistance | 0.85 | 0.002 |
| Cadmium vs. Ampicillin Resistance | 0.78 | 0.005 |
| Cadmium vs. Chloramphenicol Resistance | 0.82 | 0.003 |
| Lead vs. Tetracycline Resistance | 0.88 | 0.001 |
| Lead vs. Ampicillin Resistance | 0.81 | 0.004 |
| Lead vs. Chloramphenicol Resistance | 0.84 | 0.002 |
| Nickel vs. Tetracycline Resistance | 0.87 | 0.001 |
| Nickel vs. Ampicillin Resistance | 0.83 | 0.002 |
| Nickel vs. Chloramphenicol Resistance | 0.86 | 0.001 |

resistance to Chloramphenicol. These differences highlight the isolates' varied tolerance and resistance profiles.

Table 6 presents the correlation coefficients and p-values for the relationship between heavy metal tolerance and antibiotic resistance. Strong positive correlations are observed between Cadmium and resistance to Tetracycline ($r=0.85$), Ampicillin ($r=0.78$), and Chloramphenicol ($r=0.82$), all with significant p-values indicating robust associations. Similarly, Lead shows high correlations with resistance to Tetracycline ($r=0.88$), Ampicillin ($r=0.81$), and Chloramphenicol ($r=0.84$), all significant. Nickel also demonstrates strong correlations with Tetracycline ($r=0.87$), Ampicillin ($r=0.83$), and Chloramphenicol ($r=0.86$), with significant p-values. These results suggest a notable relationship between heavy metal tolerance and antibiotic resistance across the isolates.

The correlation and statistical analysis table shows the strength and significance of the relationship between heavy metal resistance and antibiotic resistance, with p-values indicating the statistical significance.

Table 7: Exposure to heavy metals resulted in a significant increase in antibiotic resistance in the tested bacterial isolates. *Sphingomonas paucimobilis* exhibited increased resistance, with reductions of 5 mm, 10 mm, and 4 mm in the zones of inhibition for Tetracycline, Ampicillin, and Chloramphenicol, respectively, after exposure to Cadmium. Similarly, *Acinetobacter baumannii* displayed increased resistance when exposed to lead, with decreases of 3 mm and 2 mm in the zones of inhibition for Tetracycline and Ampicillin, while no change was observed for Chloramphenicol. The most substantial increase in resistance was observed in *Pseudomonas reactans* following exposure to Nickel, where the zone of inhibition for Chloramphenicol decreased by 10 mm. additionally, the minimum inhibitory concentration (MIC) values increased across all isolates,

**Table 7** Impact of heavy metal exposure on antibiotic resistance in bacterial isolates

| Heavy Metal | Bacterial Isolate | Antibiotic | Zone of Inhibition (mm) - Control | Zone of Inhibition (mm) - Exposed | Change in Resistance | MIC (µg/mL) | Heavy Metal Exposure Concentration (µg/mL) |
|---|---|---|---|---|---|---|---|
| Cadmium (Cd) | *Sphingomonas paucimobilis* | Tetracycline | 20 | 15 | Increased Resistance (↓ 5 mm) | 35 | 10 |
| Cadmium (Cd) | *Sphingomonas paucimobilis* | Ampicillin | 32 | 22 | Increased Resistance (↓ 10 mm) | 25 | 10 |
| Cadmium (Cd) | *Sphingomonas paucimobilis* | Chloramphenicol | 22 | 18 | Increased Resistance (↓ 4 mm) | 30 | 10 |
| Lead (Pb) | *Acinetobacter baumannii* | Tetracycline | 25 | 22 | Increased Resistance (↓ 3 mm) | 25 | 15 |
| Lead (Pb) | *Acinetobacter baumannii* | Ampicillin | 21 | 19 | Increased Resistance (↓ 2 mm) | 20 | 15 |
| Lead (Pb) | *Acinetobacter baumannii* | Chloramphenicol | 24 | 24 | No Change | 15 | 15 |
| Nickel (Ni) | *Pseudomonas reactans* | Tetracycline | 23 | 17 | Increased Resistance (↓ 6 mm) | 20 | 20 |
| Nickel (Ni) | *Pseudomonas reactans* | Ampicillin | 25 | 19 | Increased Resistance (↓ 6 mm) | 12.5 | 20 |
| Nickel (Ni) | *Pseudomonas reactans* | Chloramphenicol | 25 | 15 | Increased Resistance (↓ 10 mm) | 10.5 | 20 |

Notes

MIC (µg/mL): Minimum inhibitory concentration of the antibiotic that prevents visible growth of the bacterial isolate, indicating the level of antibiotic susceptibility

Heavy Metal Exposure Concentration (µg/mL): Concentration of heavy metals used in the assay, which allows for the evaluation of their impact on antibiotic resistance

Control: Zones of inhibition for isolates not exposed to heavy metals

Exposed: Zones of inhibition for isolates exposed to heavy metals

Change in Resistance: Difference in zone diameter after exposure

further indicating heightened antibiotic resistance after heavy metal exposure. These results suggest that exposure to Cadmium, Lead, and Nickel enhances bacterial resistance to commonly used antibiotics.

## Discussion

### Impact of scrapyard contamination on soil pH and nutrient composition

The contaminated soil from the scrapyard exhibits significant differences compared to fertile agricultural soil, affecting soil health, agricultural productivity, and environmental sustainability. The higher bulk density of contaminated soil suggests potential compaction, which can restrict root growth and water infiltration, negatively impacting plant health and crop yields (Arshad et al. 1996). Although no formal assessment of vegetation in the scrapyard and its surrounding soil was conducted, the observed soil conditions—such as reduced organic matter, elevated heavy metal concentrations, and increased salinity—suggest that plant growth in these areas may be limited. These factors could hinder agricultural productivity in nearby lands and affect overall soil health. Additionally, reduced organic carbon and organic matter levels compromise soil fertility, nutrient retention, and microbial activity, leading to a diminished capacity to sustain plant life and essential microbial communities (Spohn et al. 2023; Amoakwah et al. 2024). The soil's slightly acidic pH may lead to nutrient availability issues. Soil pH is closely related to soil microbial activity and heavy-metal dissolution. Soil acidification (pH reduction) increases the dissolution rate and availability of heavy metals in soil, posing the risk of heavy-metal pollution in soil. Increasing soil pH is also an important part of soil remediation, while elevated electrical conductivity signifies higher salinity, which can cause osmotic stress and ion toxicity in plants (Barrow and Hartemink 2023). Elevated sulphate and phosphate levels pose risks for eutrophication in nearby water bodies, while lower nitrogen levels may limit plant development. Furthermore, the higher sand content and lower clay content in the contaminated soil contribute to increased drainage but reduced water retention, exacerbating issues related to nutrient availability and moisture retention.

### Heavy metal contamination and associated risks

The differences in heavy metal concentrations between scrapyard and agricultural soils highlight significant environmental and health concerns. Lead levels in scrapyard soils are markedly higher than those found in agricultural controls, suggesting that Scrapyards, often linked to metal dumping and industrial activities, contribute to elevated lead contamination (Levin et al., 2020). Lead's toxic effects, particularly on the nervous system and child development, raise concerns about the proximity of such sites to residential areas (Eigbike et al. 2024.

Similarly, cadmium concentrations in scrapyard soils are alarmingly elevated compared to control soils, emphasizing the need for monitoring and remediation efforts to mitigate exposure risks. Zinc and copper levels are also significantly higher in scrapyard soils; while both are essential micronutrients, excessive concentrations can lead to toxicity, that may affect plant growth and soil microbiota. Nickel, arsenic, and chromium concentrations are also elevated, with the latter two absent in the control soil. Both arsenic and chromium are known carcinogens that pose serious health risks. Exposure to these heavy metals has been shown to interfere with DNA synthesis and repair mechanisms, significantly increasing the risk of cancers, particularly lung cancer and other malignancies associated with environmental exposure (Smith et al. 2002; Costa and Klein 2006). Research indicates that both metals can induce oxidative stress and DNA damage, key factors in carcinogenesis (Huang et al. 2004). Additionally, even prolonged exposure to low concentrations of arsenic and chromium has been linked to an elevated risk of various cancers in humans (IARC, 2012). Additionally, increased iron concentrations in scrapyard soils may negatively influence soil fertility and microbial activity.

### Bacterial load and soil enzyme activity

The analysis of bacterial loads and soil enzyme activities reveals significant differences between scrapyard soils and control sites, providing crucial insights into the ecological impact of contamination on microbial communities. Metal-tolerant bacterial counts in scrapyard soils are markedly lower than those in the control, suggesting that metal contamination adversely affects bacterial viability and diversity. This decline indicates a stress response, limiting bacterial growth and reproduction in contaminated environments.

Soil enzyme activities serve as critical indicators of microbial metabolic processes, reflecting both intracellular and extracellular enzymatic functions that influence nutrient cycling and organic matter decomposition (Burns et al. 2013), also show notable reductions. Dehydrogenase activity suggests decreased microbial respiration and organic matter decomposition. Lower urease activity in scrapyard soils may hinder nitrogen cycling, impacting plant growth. Similarly, reduced phosphatase activity may limit phosphorus availability for plant nutrition, while lower catalase activity reflects a diminished microbial capacity to detoxify

metabolic byproducts. The findings suggest that scrapyard contamination significantly reduces bacterial loads and enzyme activities, disrupting nutrient cycling and microbial diversity, potentially leading to long-term soil degradation.

## Antibiotic and heavy metal resistance patterns in bacterial isolates

The results in Table 4 detail the resistance and sensitivity profiles of various bacterial isolates, including *Sphingomonas paucimobilis*, *Enterobacter aerogenes*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas reactans*, and *Pseudomonas putida*, against different antibiotics and heavy metals.

Significant variation in antibiotic resistance was observed. *Sphingomonas paucimobilis* and *Pseudomonas putida* exhibited resistance to ampicillin, while *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Pseudomonas reactans* remained sensitive, indicating potential therapeutic options. Resistance to tetracycline was widespread among *Sphingomonas paucimobilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Pseudomonas reactans*, raising concerns about treatment effectiveness. Similar resistance patterns were noted for chloramphenicol.

Regarding heavy metals, *Sphingomonas paucimobilis* and *Pseudomonas putida* showed resistance to cadmium, while *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Pseudomonas reactans* were sensitive. Notably, *Sphingomonas paucimobilis* and *Klebsiella pneumoniae* were sensitive to lead, whereas *Enterobacter aerogenes*, *Pseudomonas putida*, and *Pseudomonas reactans* exhibited resistance, suggesting their adaptability to contaminated environments. Resistance to nickel was particularly notable in *Sphingomonas paucimobilis*, *Pseudomonas putida*, and *Pseudomonas reactans*, indicating the presence of unique metabolic pathways or efflux mechanisms that could be harnessed for bioremediation applications.

## Bacterial community structure and adaptation mechanisms

The identification of bacterial isolates from metal-contaminated soils using 16 S rRNA gene sequencing has provided significant insights into microbial community structure. The predominant genera identified were *Pseudomonas reactans* (50%), *Bacillus subtilis* (30%), and *Acinetobacter baumannii* (20%). This distribution highlights their adaptability and potential roles in bioremediation.

The dominance of *Pseudomonas reactans* is notable due to its metabolic versatility, allowing it to survive in metal-contaminated environments. *Bacillus subtilis* is also recognized for its plant growth-promoting properties and heavy metal tolerance, contributing to phytoremediation by improving plant resilience to metal stress (Ramírez et al. 2019; Wróbel et al. 2023). *Acinetobacter baumannii*, while less dominant, plays a role in pollutant degradation and biosorption of heavy metals. The interaction between these bacteria may enhance bioremediation strategies.

## Correlation between heavy metal tolerance and antibiotic resistance

The relationship between heavy metal tolerance and antibiotic resistance is crucial for understanding microbial adaptation in contaminated environments. Data from Table 6 indicate strong positive correlations between heavy metal exposure and antibiotic resistance, suggesting that increased tolerance to cadmium, lead, and nickel corresponds with heightened resistance to specific antibiotics.

Lead exposure was found to enhance antibiotic resistance, aligning with studies indicating that bacteria exposed to heavy metals often acquire resistance mechanisms conferring tolerance to both metals and antibiotics. Similarly, correlations between nickel tolerance and increased antibiotic resistance suggest a shared genetic basis for these traits (Wright et al. 2006; Ejaz et al. 2022). The co-selection of antibiotic resistance alongside heavy metal tolerance has significant public health implications. Bacteria adapting to polluted environments may become reservoirs for multidrug-resistant pathogens, posing risks for food safety and disease transmission (Van Duin and Paterson 2016).

## Implications for environmental management and public health

Table 7 highlights the impact of heavy metal exposure on antibiotic resistance. The findings indicate that heavy metal exposure significantly enhances antibiotic resistance in bacterial isolates, reinforcing concerns that environmental contamination contributes to the rise of antimicrobial resistance.

For *Sphingomonas paucimobilis*, cadmium exposure resulted in reduced antibiotic susceptibility, suggesting that metal-induced selection pressure may activate resistance mechanisms such as efflux pumps or altered membrane permeability (Abbas et al. 2014). Similarly, *Acinetobacter baumannii* exhibited increased resistance following lead exposure, while *Pseudomonas reactans* showed a significant reduction in the zone of inhibition for chloramphenicol after nickel exposure.

Resistant bacteria play a crucial role in reducing heavy metal bioavailability in soil through multiple mechanisms. These include the adsorption of soluble heavy metals and antibiotics, the transformation of highly reactive metal species into less bioavailable forms, and the enzymatic

degradation of soil antibiotics. These processes collectively contribute to the detoxification of contaminated environments, thereby mitigating the ecological and health risks associated with heavy metal and antibiotic pollution.

The observed increase in minimum inhibitory concentration (MIC) across all isolates suggests that environmental contamination with cadmium, lead, and nickel contributes to the emergence of resistant bacterial strains. These findings underscore the need for stricter regulations on heavy metal pollution and further research into the interplay between environmental contaminants and antimicrobial resistance.

Collectively, these data suggest that exposure to heavy metals enhances antibiotic resistance among bacterial isolates, posing significant public health risks as antibiotic-resistant infections become increasingly difficult to treat. The co-selection of antibiotic and heavy metal resistance creates environments where pathogenic bacteria thrive, resulting in higher treatment failures and prolonged illness (Seiler and Berendonk 2012; Pal et al. 2015). Furthermore, the persistence of heavy metals in the environment exacerbates drug-resistant bacteria spread and endangers human health, necessitating stricter regulations on heavy metal discharges.

**Acknowledgements** We appreciate the assistance of our colleagues and the laboratory staff for their valuable help during the research process.

**Author contributions** ATA designed the study JFA and AZB performed the experiment AWT and ATA analyzed the data ATA and JFA wrote the manuscript All authors read and approved the final manuscript.

**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Competing interests** The authors declare no competing interests.

## References

Abbas SZ, Rafatullah M, Ismail N, Lalung J (2014) Isolation, identification, and characterization of cadmium resistant *Pseudomonas* Sp. M3 from industrial wastewater. J Waste Manage 2014(1):160398

AL-Sa'ady AR, Shaker W, Salam M (2014) Antibiotic susceptibility and heavy metal tolerance pattern of *Staphylococcus epidermidis*, and curing of plasmid. Curr Res Microbiol Biotechnol 2:402–405

Amarasekara NR, Mafiz AI, Qian X, Tiedje JM, Hao W, Zhang Y (2023) Exploring the co-occurrence of antibiotic, metal, and biocide resistance genes in the urban agricultural environment. J Agric Food Res 11:100474

Amoakwah E, Kim SH, Jeon S, Shim JH, Lee YH, Kwon SI, Park SJ (2024) Long-term fertilization and liming increase soil fertility but reduce carbon stratification and stocks of paddy rice soils. Front Soil Sci 4:1426894

Amos IA, Edward BA, Maryam I (2023) Quality assessment of the physicochemical properties of soils from automobile workshops and transformer installations site in Lafia, Nasarawa state. Sci World J 18(1):20–25

Angelina E, Papatheodorou EM, Demirtzoglou T, Monokrousos N (2020) Effects of *Bacillus subtilis* and *Pseudomonas fluorescens* inoculation on attributes of the lettuce (Lactuca sativa L.) soil rhizosphere microbial community: the role of the management system. Agronomy 10(9):1428

Aprile F, Lorandi R (2012) Evaluation of cation exchange capacity (CEC) in tropical soils using four different analytical methods. J Agric Sci 4(6):278

Arshad MA, Lowery B, Grossman B (1996) Physical Tests for Monitoring Soil Quality. In: Doran J.W., Jones A.J., editors. Methods for assessing soil quality. Madison, WI. p 123–41

Ayalew F (2021) Antimicrobial Activity Of *Actinomyctes* And Drug Resistance Profile of Common Bacteria Isolated from Municipal Waste Dump Site Inbahir Dar City, Ethiopia (Doctoral dissertation)

Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV (2006) Co-selection of antibiotic and metal resistance. Trends Microbiol 14(4):176–182

Barbosa FG, Marcelino PRF, Lacerda TM, Philippini RR, Giancaterino ET, Mancebo MC, da Silva SS (2022) Production, physicochemical and structural characterization of a bio emulsifier produced in a culture medium composed of sugarcane bagasse hemicellulosic hydrolysate and soybean oil in the context of bio refineries. Fermentation 8(11):618

Barbosa JZ, Poggere GC, Teixeira WWR, Motta ACV, Prior SA, Curi N (2020) Assessing soil contamination in automobile scrap yards by portable X-ray fluorescence spectrometry and magnetic susceptibility. Environ Monit Assess 192(1):46

Barrow NJ, Hartemink AE (2023) The effects of pH on nutrient availability depend on both soils and plants. Plant Soil 487(1):21–37

Basatian-Tashkan B, Niakan M, Khaledi M, Afkhami H, Sameni F, Bakhti S, Mirnejad R (2020) Antibiotic resistance assessment of *Acinetobacter baumannii* isolates from Tehran hospitals due to the presence of efflux pumps encoding genes (adeA and AdeS genes) by molecular method. BMC Res Notes 13:1–6

Bilgili AV, Van Es HM, Akbas F, Durak A, Hively WD (2010) Visible-near infrared reflectance spectroscopy for assessment of soil properties in a semi-arid area of Turkey. J Arid Environ 74(2):229–238

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN (2013) Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol Biochem 58:216–234

Chen J, Li J, Zhang H, Shi W, Liu Y (2019) Bacterial heavy-metal and antibiotic resistance genes in a copper tailing dam area in Northern China. Front Microbiol 10:1916

Chen LK, Kuo SC, Chang KC, Cheng CC, Yu PY, Chang CH, Tseng CC (2017) Clinical antibiotic-resistant *Acinetobacter baumannii* strains with higher susceptibility to environmental phages than antibiotic-sensitive strains. Sci Rep 7(1):6319

Chukwu KB, Abafe OA, Amoako DG, Essack SY, Abia AL (2023) Environmental concentrations of antibiotics, biocides, and heavy metals fail to induce phenotypic antimicrobial resistance in *Escherichia coli*. Sci Total Environ 899:165721

Clinical and Laboratory Standards Institute (2018) Performance standards for antimicrobial susceptibility testing; 27th informational supplement. M100-S28. Clinical and Laboratory Standards Institute, Wayne, PA

Costa M, Klein CB (2006) Toxicity and carcinogenicity of chromium compounds in humans. Crit Rev Toxicol 36(2):155–163

Edet UO, Bassey IU, Joseph AP (2023) Heavy metal co-resistance with antibiotics amongst bacteria isolates from an open dumpsite soil. Heliyon, *9*(2)

Eigbike CO, Odion NS, Ojo-Igbinosa U (2024) Assessment of heavy metal contamination of some designated scrap-yards in Benin City, Edo State, Nigeria. Afr J Health Saf Environ 5(1):1–10

Ejaz H, Junaid K, Yasmeen H, Naseer A, Alam H, Younas S, Bukhari SNA (2022) Multiple antimicrobial resistance and heavy metal tolerance of biofilm-producing bacteria isolated from dairy and non-dairy food products. Foods 11(18):2728

Engin AB, Engin ED, Engin A (2023) Effects of co-selection of antibiotic-resistance and metal-resistance genes on antibiotic-resistance potency of environmental bacteria and related ecological risk factors. Environ Toxicol Pharmacol 98:104081

Fawole MO, Oso BA (2001) Laboratory manual of microbiology. Rev. Spectrum Books, ed. Ibadan

Gillieatt BF, Coleman NV (2024) Unravelling the mechanisms of antibiotic and heavy metal resistance co-selection in environmental bacteria. FEMS Microbiol Rev, fuae017

Goff JL, Chen Y, Thorgersen MP, Hoang LT, Poole FL, Szink EG, Adams MW (2023) Mixed heavy metal stress induces global iron starvation response. ISME J 17(3):382–392

Guilin W (2015) The research of scrapped automobiles recycling and disassembling industry development based on auto Industry chain. In *MATEC Web of Conferences* (Vol. 26, p. 03004). EDP Sciences

Huang C, Ke Q, Costa M, Shi X (2004) Molecular mechanisms of arsenic carcinogenesis. Mol Cell Biochem 255(1–2):57–66

Ibe FC, Opara AI, Ibe BO (2020) Application of pollution risk evaluation models in groundwater systems in the vicinity of automobile scrap markets in Owerri municipal and environs, southeastern Nigeria. Sci Afr 8:e00450

International Agency for Research on Cancer (IARC) (2012) Arsenic, metals, fibres, and dusts (Vol. 100 C). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*

Jeong SW, Choi YJ (2020) Extremophilic microorganisms for the treatment of toxic pollutants in the environment. Molecules 25(21):4916

Levin,R., Vieira, C. L. Z., Mordarski, D. C., & Rosenbaum, M. H. (2020). Leadseasonality in humans, animals, and the natural environment. Environmentalresearch, 180, 10879

Levin R, Zilli Vieira CL, Rosenbaum MH, Bischoff K, Mordarski DC, Brown MJ (2021) The urban lead (Pb) burden in humans, animals, and the natural environment. Environ Res 193:110377

Lin H, Jiang L, Li B, Dong Y, He Y, Qiu Y (2019) Screening and evaluation of heavy metals facilitating antibiotic resistance gene transfer in a sludge bacterial community. Sci Total Environ 695:133862

Li T, Meng L, Herman U, Lu Z, Crittenden J (2015) A survey of soil enzyme activities along major roads in Beijing: the implications for traffic corridor green space management. Int J Environ Res Public Health 12:12475–12488

Liu T, Ni SY, Wang Z (2024) Contamination and health risk assessment of heavy metals in soil surrounding an automobile industry factory in Jiaxing, China. Front Environ Sci 12:1362366

Liu Y, Tan H (2014) Changes of growth and nutrient-relating enzymatic activities of sargassum thunbergii when exposed to different nutrient conditions. Aquat Sci Technol 2:1–13

Maurya AP, Rajkumari J, Bhattacharjee A, Pandey P (2020) Development, spread and persistence of antibiotic resistance genes (ARGs) in the soil microbiomes through co-selection. Rev Environ Health 35(4):371–378

Minarini LADR, Andrade LND, De Gregorio E, Grosso F, Naas T, Zarrilli R, Camargo IL (2020) Antimicrobial resistance as a global public health problem: how can we address it? Front Public Health 8:612844

Mlinarics E, Dergez Á, Blaskó L, Bordás D, Zsigrai G, Kiss I, Szabó S (2009) Possibilities of plant production for biorefinery on a soil contaminated with heavy metals. Analele Univ Tßii Din Oradea Fascicula: Protectßia Mediului 14:746–753

Mustapha MU, Halimoon N (2015) Screening and isolation of heavy metal tolerant bacteria in industrial effluent. Procedia Environ Sci 30:33–37

Najar IN, Das S, Kumar S, Sharma P, Mondal K, Sherpa MT, Thakur N (2022) Coexistence of heavy metal tolerance and antibiotic resistance in thermophilic bacteria belonging to genus *Geobacillus*. Front Microbiol 13:914037

Nannipieri P, Trasar-Cepeda C, Dick RP (2018) Soil enzyme activity: A brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. Biol Fertil Soils 54(1):11–19

Nath S, Paul P, Roy R, Bhattacharjee S, Deb B (2019) Isolation and identification of metal-tolerant and antibiotic-resistant bacteria from soil samples of Cachar district of Assam, India. SN Appl Sci 1(7):727

Ntabugi KMM, Manegabe BJ, Dewar JB, Simbahan JF, Flavier ME, Sekomo CB (2021) Synergistic increase in antibiotic resistance with tolerance to cadmium and lead in environmental bacteria isolated from the San Cristobal river, Laguna de Bay, Philippines. Int J Environ Stud 78(1):165–183

Pal A, Bhattacharjee S, Saha J, Sarkar M, Mandal P (2022) Bacterial survival strategies and responses under heavy metal stress: A comprehensive overview. Crit Rev Microbiol 48(3):327–355

Pal C, Asiani K, Arya S, Rensing C, Stekel DJ, Larsson DJ, Hobman JL (2017) Metal resistance and its association with antibiotic resistance. Adv Microb Physiol 70:261–313

Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DJ (2015) Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics 16:1–14

Prabhakaran P, Ashraf MA, Aqma WS (2016) Microbial stress response to heavy metals in the environment. RSC Adv 6(111):109862–109877

Ramírez V, Baez A, López P, Bustillos R, Villalobos MÁ, Carreño R, Munive JA (2019) Chromium hyper-tolerant Bacillus Sp. MH778713 assists phytoremediation of heavy metals by mesquite trees (Prosopis laevigata). Front Microbiol 10:1833

Seiler C, Berendonk TU (2012) Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front Microbiol 3:399

Smith AH, Lingas EO, Rahman M (2002) Contamination of drinking-water by arsenic in Bangladesh: A public health emergency. Bull World Health Organ 78(9):1093–1103

Spohn M, Braun S, Sierra CA (2023) Continuous decrease in soil organic matter despite increased plant productivity in an 80-years-old phosphorus-addition experiment. Commun Earth Environ 4:251. https://doi.org/10.1038/s43247-023-00915-1

Talat A, Khan F, Khan AU (2024) Genome analyses of colistin-resistant high-risk Bla NDM-5 producing *Klebsiella pneumoniae* ST147 and *Pseudomonas aeruginosa* ST235 and ST357 in clinical settings. BMC Microbiol 24(1):174

Tan X, Xie B, Wang J, He W, Wang X, Wei G (2014) County-scale Spatial distribution of soil enzyme activities and enzyme activity indices in agricultural land: implications for soil quality assessment. Sci World J 2014(1):535768

Van Duin D, Paterson DL (2016) Multidrug-resistant bacteria in the community: trends and lessons learned. Infect Disease Clin 30(2):377–390

Vats P, Kaur UJ, Rishi P (2022) Heavy metal-induced selection and proliferation of antibiotic resistance: A review. J Appl Microbiol 132(6):4058–4076

Wang X, Lan B, Fei H, Wang S, Zhu G (2021) Heavy metal could drive co-selection of antibiotic resistance in terrestrial subsurface soils. J Hazard Mater 411:124848

Wróbel M, Śliwakowski W, Kowalczyk P, Kramkowski K, Dobrzyński J (2023) Bioremediation of heavy metals by the genus Bacillus. Int J Environ Res Public Health 20(6):4964

Wright MS, Peltier GL, Stepanauskas R, McArthur JV (2006) Bacterial tolerances to metals and antibiotics in metal-contaminated and reference streams. FEMS Microbiol Ecol 58(2):293–302

Yao H, Liu J, Jiang X, Chen F, Lu X, Zhang J (2021) Analysis of the clinical effect of combined drug susceptibility to guide medication for carbapenem-resistant Klebsiella pneumoniae patients based on the kirby–bauer disk diffusion method. Infect Drug Resist, 79–87

Yeboah JO, Shi G, Shi W (2021) Effect of heavy metal contamination on soil enzymes activities. J Geoscience Environ Prot 9(6):135–154

Zhou S, Xiong C, Su Y, Wang Y, Gao Y, Tang Z, Duan Y (2022) Antibiotic-resistant bacteria and antibiotic resistance genes in uranium mine: distribution and influencing factors. Environ Pollut 304:119158

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.